hours of heating a sample from the flask was poured into water and the resulting precipitate gave a melting point of 120°. Another 34 g. of 30% hydrogen peroxide was added and the flask left standing for seven days. The mixture was then poured into water and the precipitate was removed by filtration, washed with water, dried and crystallized from alcohol, yield 50 g. (96.3%), m. p. 218.5–219°. *Anal.* Calcd. for $C_{22}H_{18}O_2S$: C, 76.26; H, 5.23. Found: C, 76.53; H, 5.45.

The sulfone was slightly soluble in cold alcohol and benzene; and soluble in chloroform, acetone, hot alcohol, hot toluene, hot benzene and hot acetic acid.

p-Nitrobenzylbenzyl Sulfide.—In a 250-ml. Erlenmeyer flask was placed 34.3 g. (0.2 mole) of *p*-nitrobenzyl chloride, 24.82 g. (0.2 mole) of benzylmercaptan and 75 ml. of absolute alcohol. The flask was heated to about 50° and a slow stream of ammonia gas from a cylinder was introduced with intermittent cooling. The solution turned green, followed by the immediate precipitation of white crystals. The ammonia was introduced for thirty minutes, the precipitate collected; the filtrate on standing gave additional crystals, yield 49.2 g. (95%), m. p. 56–57°. *Anal.* Calcd. for $C_{14}H_{13}NO_2S$: N, 5.40. Found: N, 5.20.

Ultraviolet Absorption Spectrum.—The ultraviolet absorption spectrum of I was determined with a Beckmann quartz ultraviolet spectrophotometer with 95% ethanol as the solvent. The spectrum is shown in Fig. 1 in which the molar extinction coefficients are plotted against the wave lengths in ångström units.

Summary

1. *p,p'*-Dichlorobenzyl sulfoxide and sulfone, β-naphthylmethyl sulfide and sulfone, α-naphthylmethyl sulfide and sulfone, and *p*-nitrobenzylbenzyl sulfide have been prepared and characterized.

2. The condensation of ethyl oxalate and three arylmethyl sulfones has been described. Evidence has been presented to show that the product from benzyl sulfone and ethyl oxalate is a 2,5-diaryl-3,4-dihydroxythiophene-1-dioxide which exhibits keto–enol tautomerism.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

Acetylative Desulfation of Carbohydrate Acid Sulfates[1]

BY M. L. WOLFROM AND REX MONTGOMERY[2]

Progress in the determination of the structure of heparin and related sulfated polysaccharides has been impeded by the lack of a suitable desulfation procedure. We report herein a method whereby the sulfated polysaccharide is dissolved at low temperatures in essentially absolute sulfuric acid. When the resultant solution is treated with a large excess of acetic anhydride, the acetylated and desulfated product is obtained and can readily be isolated by convenient methods. It is desirable to answer two questions regarding this procedure. First, is Walden inversion involved and, second, is the reaction accompanied by significant glycosidic hydrolysis? The former question can be answered in the negative since the desulfation of D-glucose 3-sulfate and D-glucose 6-sulfate led to the isolation of α-D-glucopyranose pentaacetate. The extent of glycosidic hydrolysis is, however, dependent upon the nature of the glycosidic linkage, being negligible in the case of cellobiose and natural trehalose, but predominant in gentiobiose and melezitose (Table I). Sugars containing a fructofuranose residue were hydrolyzed with apparent destruction of the fructose portion and the acetylation of the remainder of the molecule. Thus sucrose gave α-D-glucopyranose pentaacetate. It is also of interest to note that when levoglucosan (1,6-anhydro-β-D-glucopyranose) was subjected to this acetylation procedure, α-D-glucopyranose pentaacetate was

(1) The data herein recorded supersede those reported by M. L. Wolfrom and F. A. H. Rice, *Abstracts Papers Am. Chem. Soc.*, **113**, 1Q (1948).

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TABLE I

ACETYLATION[a] OF SUGARS AND SUGAR DERIVATIVES WITH ACETIC ANHYDRIDE IN HYDROGEN SULFATE

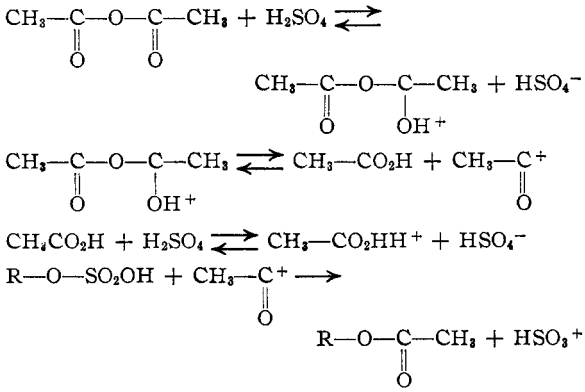
Substance	Product[b]	Yield, %
1,2:5,6-Diisopropylidene-D-glucose	α-D-Glucopyranose pentaacetate	19
Sucrose	α-D-Glucopyranose pentaacetate	44–65
Melezitose	α-D-Glucopyranose pentaacetate	30
Gentiobiose	α-D-Glucopyranose pentaacetate[f]	37
Levoglucosan (1,6-anhydro-β-D-glucopyranose)	α-D-Glucopyranose pentaacetate	28
α-D-Glucose	α-D-Glucopyranose pentaacetate	53
D-Glucosamine hydrochloride[c]	Pentaacetyl-α-D-glucosamine[d]	51
	Pentaacetyl-β-D-glucosamine[d]	2
β-Cellobiose	α-Cellobiose octaacetate	41–45
Stachyose	α-D-Glucopyranose pentaacetate[f]	40
Raffinose	α-D-Glucopyranose pentaacetate[f]	24
α-D-Galactose	α-D-Galactopyranose pentaacetate[g]	20
Trehalose (natural)	Trehalose octaacetate	86
D-Mannitol	D-Mannitol hexaacetate[e]	84

[a] All substances were finely powdered and were dried under reduced pressure at 75–80° over phosphorus pentoxide. The acetylation procedure was that described for the simultaneous desulfation and acetylation of D-glucose 6-sulfate and the products were isolated in the same manner. The solutions of the sugars in hydrogen sulfate were nearly colorless save for those containing D-fructose which were initially brown and developed a green fluorescence in the acetylation mixture. Like results were also obtained on adding the solids to a similarly cooled mixture of hydrogen sulfate and acetic anhydride (1:10 by vol.). [b] Identification by melting point, mixed melting point and chloroform rotation. [c] Dissolved readily in the reaction mixture. [d] From ethanol–ether. [e] The product was crystalline and pure without recrystallization. [f] The compound was isolated from the sirupy reaction product by chromatographic analysis, as per W. H. McNeely, W. W. Binkley and M. L. Wolfrom, THIS JOURNAL, 67, 527(1945). [g] The product did not crystallize for many months.

obtained, indicating the instability under these conditions of 1,6-anhydro rings.

When this procedure was applied to sodium heparinate there was obtained a reducing acetate that was by reaction an acid and which was free of sulfur. It gave positive color tests for uronic acid and hexosamine. Except for some indication of lactone structure, analogous results were obtained with the sodium salts of chondroitinsulfuric acid (from cartilage) and mucoitinsulfuric acid (from pig gastric mucin). The fact that these acetylated and desulfated polysaccharides exhibited significant Fehling reduction indicates that again some glycosidic hydrolysis occurred and the products must consequently be considered to be acetates of the degraded parent polysaccharides. The extent of degradation has not been determined but these products are under further investigation.

It is of interest to speculate upon the possible nature of the desulfation reaction. The formation of intermediate sulfate acid esters and their replacement by acetate groups has long been postulated in the commercial processes employed in the manufacture of cellulose acetate[3,4] and has been well characterized recently.[5] The use of absolute sulfuric acid or hydrogen sulfate as a solvent for organic compounds has been rather extensively investigated.[6-9] Although the reaction described herein required a large excess of acetic anhydride and hence the solvent is not hydrogen sulfate alone, it is probable that, following Kuhn,[10] this ester exchange reaction proceeds as shown.



In the model experiments involving the simultaneous desulfation and acetylation of reducing sugar sulfates, the α-D forms of the aldoses were the principal products obtained. This was to be expected from the well-known α-directing influence of acid acetylation catalysts. The yields obtained from the reducing sugars by this technic were not very high: D-glucose, D-galactose, D-glucosamine hydrochloride and cellobiose (Table I). With cellobiose the yield of its α-acetate was of the same order as with D-glucose. Better yields were found for the non-reducing structures D-mannitol and trehalose. That for D-galactose was low and its pentaacetate was not isolated from the D-galactose-containing oligosaccharides raffinose and stachyose, a behavior probably related to the established tendency of this sugar to react in more than one ring form.

Experimental

Simultaneous Desulfation and Acetylation of D-Glucose 6-Sulfate and D-Glucose 3-Sulfate.—Equal volumes of fuming sulfuric acid (30% SO_3) and concentrated sulfuric acid (sp. gr. 1.84/60°F.) were mixed with cooling in a sodium chloride–ice–water-bath and concentrated sulfuric acid was added until the mixture melted at 10.5°. Additional concentrated sulfuric acid was then added until the melting point of the solution was between 7 and 9°. The resulting acid was somewhat less than absolute.

To 5 ml. of the above acid, in the form of a frozen powder, was added 0.85 g. of the finely powdered, amorphous sodium salt of D-glucose 6-sulfate, prepared essentially according to the general procedure of Percival and Soutar.[11] The salt had been previously dried overnight at 80° under reduced pressure and over phosphorus pentoxide. The salt was added to the powdered acid and the two solids thoroughly mixed at −15 to −20° (sodium chloride–ice-water) while maintaining an inert atmosphere of carbon dioxide. Depression of the freezing point of the acid by the salt eventually produced a viscous solution which was allowed to stand at −15 to −20° for one to two hours. An amount of 50 ml. of acetic anhydride was added with stirring at such a rate as to maintain the temperature of the solution below −10°. The addition of the first 5 ml. required one to two hours and the remainder was added over a period of about four to six hours. Throughout these additions the atmosphere of carbon dioxide was maintained in order to prevent the ingress of moisture. After the addition of acetic anhydride, the reaction flask was tightly stoppered and maintained at −15 to −20° for fourteen to sixteen hours and then at room temperature (20–25°) for about four hours after which it was slowly added to an ice–water–sodium acetate mixture (produced by adding 18 g. of anhydrous sodium acetate to about 200 g. of crushed ice) so that the temperature never exceeded −10°. The resultant solution was immediately extracted with five 50-ml. portions of chloroform and the combined extracts were dried with Drierite (anhydrous calcium sulfate). The dried chloroform solution was concentrated to dryness under reduced pressure at 40° and the residue (0.90 g.) was crystallized once from 95% ethanol; m. p. 110–111°, $[\alpha]^{22}D$ + 100° (*c* 0.8, chloroform). The melting point was not depressed on admixture with α-D-glucopyranose pentaacetate for which Hudson and Dale[12] cite: m. p. 112–113° (cor.), $[\alpha]^{20}D$ + 102° (*c* 5.25, chloroform).

The amorphous sodium salt of D-glucose 3-sulfate[13] and of 1,2:5,6-diisopropylidene-D-glucose 3-sulfate[13] likewise yielded α-D-glucopyranose pentaacetate in 32 and 65% yield, respectively, when treated according to the proce-

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(13) H. Ohle, *Biochem. Z.*, **136**, 428 (1923); E. G. V. Percival, *J. Chem. Soc.*, 119 (1945).

dure described above for the sodium salt of D-glucose 6-sulfate.

Simultaneous Desulfation and Acetylation of Sodium Heparinate.—An amount of 2 g. of finely ground and dried (at 80° and under reduced pressure over phosphorus pentoxide) amorphous sodium heparinate, prepared through the benzidine and the crystalline barium acid salts, was added under an atmosphere of carbon dioxide to 20 ml. of frozen, powdered, nearly absolute sulfuric acid (prepared as described above for the simultaneous desulfation and acetylation of D-glucose 6-sulfate). The mixture was maintained at −10 to 0° with occasional stirring for a period of two to three hours after which time all but a small amount of material (0.16 g.) was in solution. The resulting solution was light brown in color and was cooled in a sodium chloride–ice–water-bath before adding the acetic anhydride. An amount of 40 ml. of acetic anhydride was then added dropwise with continual stirring, the rate of addition being such that the temperature never exceeded −10°. The solution became very viscous after about one volume of acetic anhydride had been added but became quite mobile after the addition of about two volumes. Similarly, the heat of reaction increased and then became negligible after about two volumes of acetic anhydride had been added. An additional 140 ml. of acetic anhydride was then added dropwise over a period of five to six hours with mechanical stirring, the temperature being maintained at about −15°. An atmosphere of carbon dioxide was maintained throughout the additions, after which the flask was tightly stoppered. After standing overnight at −15°, the reaction mixture was brought to room temperature for two to three hours and the solution decanted from any undissolved sodium heparinate. The solution was added dropwise to sodium acetate–ice–water (produced by adding 70 g. of anhydrous sodium acetate to about 300 g. of crushed ice) at a rate such that the temperature never exceeded −10°. The resulting solution was extracted with five 100-ml. portions of chloroform, great care being taken to avoid any emulsification, by very careful shaking, since prolonged solution of the heparin acetate in the aqueous medium resulted in a low yield. The dried (with Drierite) chloroform extract was evaporated to dryness under 1 mm. pressure and the residue was dissolved in 10 ml. of chloroform. The heparin acetate was precipitated by adding the chloroform solution dropwise and with stirring to 200 ml. of petroleum ether (b. p. 30–60°). The precipitated light brown amorphous solid was filtered and washed with ether; yield 1.69 g. The product was appreciably reducing to Fehling solution. It exhibited an acid reaction and a positive color test for uronic acid[14] and hexosamine.[15] Sulfur was absent. It was soluble in water, chloroform, ethanol, methanol and acetone but was insoluble in ether and petroleum ether. Acetyl was present. The further characterization of this product is reserved for a later communication.

Simultaneous Desulfation and Acetylation of Sodium Chondroitinsulfate and Sodium Mucoitinsulfate.—An amount of 2.0 g. of sodium chondroitinsulfate[16] from cartilage was treated as described above for sodium heparinate and the product, a nearly colorless, amorphous solid, was isolated in the same manner; yield 0.54 g., uronic acid color test positive, hexosamine color test positive, sulfur absent, acetyl present. The product was soluble in chloroform, methanol, ethanol, water and acetone but was insoluble in ether and petroleum ether. Its aqueous solution was neutral to litmus and was strongly reducing to Fehling solution.

Sodium mucoitinsulfate (S, 3%) was prepared from pig gastric mucin by the previously described method[16] except that the treatment with Lloyd's reagent was omitted. An amount of 0.89 g. was treated as described above for sodium heparinate. The resulting solution in acetic anhydride and sulfuric acid was dark green after standing overnight and a green fluorescence was obvious as in the case of sucrose. Isolation of the product was carried out as described for sodium heparinate; yield 0.17 g., amorphous solid, nearly colorless, reducing to Fehling solution, positive color tests for uronic acid and hexosamine, acetyl present, sulfur absent. The product was soluble in chloroform, methanol, ethanol, acetone and water but was insoluble in diethyl ether and petroleum ether. Its aqueous solution was neutral to litmus.

The further characterization of these two products is reserved for a subsequent communication.

Acknowledgment.—The sodium heparinate used in this work was kindly furnished by Hoffmann–La Roche, Inc., of Nutley, New Jersey. The sodium chondroitinsulfate and sodium mucoitinsulfate were prepared in these laboratories by Mr. R. K. Madison and Mr. G. Sutherland, respectively.

Summary

1. A procedure employing absolute sulfuric acid solutions has been devised for the simultaneous desulfation and acetylation of carbohydrate acid sulfates ($ROSO_2OH$) without Walden inversion but with some glycosidic hydrolysis.

2. Application of the procedure to the sodium salts of heparinic, chondroitinsulfuric and mucoitinsulfuric acids leads to the isolation of degraded, acetylated products under further investigation.

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